Production of wood decay enzymes, mass loss and lignin solubilization in wood by marine ascomycetes and their anamorphs

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A study was carried out to establish the wood decay ability for a large number of diverse marine ascomycetes and their anamorphs. *In vitro* production of cellulase and xylanase was widespread among forty-seven fungi. Production of enzymes involved in lignin degradation was comparatively less common. Most isolates were capable of causing mass loss in a birch wood substrate although values were low (<5%) during a 24-week period. A few ascomycetes caused higher mass loss of up to 20.1%. In all cases wood decay was greater in exposed rather than submerged conditions. *Ascocratera manglicola*, *Astrosphaeriella striatispora*, *Cryptovalsa halosarceicola*, *Linocarpon bipolaris* and *Rhizophila marina*, were shown to solubilize significant amounts of lignin, with indices of lignin solubilization comparable to those of terrestrial white-rot basidiomycetes. Certain marine ascomycetes may therefore fulfill an equivalent ecological role.

Key words: marine fungi; wood decay; lignin

Introduction

Lignocellulosic substrates in the marine environment, particularly mangrove wood, support a diverse mycota (Sarma and Hyde, 2001). Most species described to date are ascomycetes, with fewer anamorphic states known. Very few basidiomycetes are known, with many of the latter also encountered infrequently (Jones and Alias, 1997; Sarma and Vittal, 2001). Although marine borers are recognized as particularly aggressive wood degraders in marine waters, they are not active in the upper intertidal zone and so fungi are thought to be important wood degraders in this niche (Hyde,

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1991). Bacterial wood decay in marine environments is relatively slow and superficial (Singh and Butcher, 1991).

The degradation mechanisms of wood by terrestrial fungi are well known and it is assumed similar mechanisms exist in marine fungi (Pointing and Hyde 2000). Cellulose hydrolysis is achieved by endoglucanases and cellobiohydrolases, collectively termed cellulases (Eaton and Hale, 1993). Hydrolysis of hemicellulose, a mixed polymer, occurs via the action of hydrolytic xylanases, mananases and possibly other hydrolases with broad substrate specificity (Eaton and Hale, 1993). The mineralization of lignin involves two peroxidases, lignin peroxidase and Mn-dependant peroxidase, and a polyphenoloxidase, laccase, collectively known as lignin-modifying enzymes (LME's). These enzymes catalyze production of highly reactive radicals which oxidize phenolic and non-phenolic lignin components (Pointing, 2001).

Three fungal wood decay types are recognized (Eaton and Hale, 1993). Soft rot, a superficial decay where enzymatic decay of cellulose and hemicellulose in surface layers of wood is accompanied by little or no lignin degradation. This is characteristic of many ascomycetes and their anamorphs. White rot, where rapid and extensive decay of all wood components due to enzymatic degradation is observed, with characteristic wood bleaching as a result of lignin removal. White-rot decay has been identified to date only among basidiomycetes and a few higher ascomycete genera (Risna and Suhirman, 2002; Urairuj *et al*., 2003). The role of white-rot fungi in lignocellulose turnover is key since lignin is the most recalcitrant component of wood. Brown rot, in which very rapid cellulose and hemicellulose decay is attributed to non-enzymatic oxidation with little or no associated lignin degradation. Certain marine fungi are known to cause soft rot (Leightley and Eaton 1979a, Mouzouras 1986, 1989a) and white rot (Leightley and Eaton 1979b, Mouzouras 1986, 1989b) decay, although studies have generally focused on taxa isolated from man-made wooden structures in temperate locations. Brown rot fungi are not colonizers of very wet or waterlogged wood and so are not found in marine environments.

The major gaps in our knowledge of marine fungal wood decay relate firstly to the lack of data relating to enzyme production by species common to natural substrates such as mangrove wood in the tropics. Secondly whether they are significant wood degraders under conditions where they occur, such as the intertidal region of mangroves. Thirdly it is not clear if marine fungi play a significant role in lignin breakdown since basidiomycetes, conventionally regarded as key lignin degraders in terrestrial environments, are rarely encountered. The aim of this study is to improve our knowledge in these areas. Firstly, by screening those fungi commonly encountered in tropical and sub-

tropical marine habitats for *in vitro* production of wood decay enzymes. Secondly, to establish the ability of such fungi to effect mass loss in wood during simulated exposed (high tide) and submerged (low tide) conditions. Thirdly, the ability of selected taxa to solubilize lignin from wood is assessed in an effort to understand the extent to which fungal lignin degradation may be achieved by marine ascomycetes and their anamorphs.

Materials and Methods

Organisms and culture conditions

Forty-five marine fungal taxa, with many commonly occurring in mangroves (Sarma and Hyde, 2001), were obtained from The University of Hong Kong Culture Collection or City University of Hong Kong Culture Collection. Reference fungi were obtained from CABI Bioscience UK. Cultures were maintained on potato-dextrose agar (Difco) supplemented with 1.5% (w/v) marine salts (Instant Ocean) at 25°C in darkness. Inoculum for enzyme production assays and wood decay testing consisted of 5mm agar plugs cut from the actively growing colony margin of 4-8 week cultures of each fungus. Single agar plugs were used for agar based enzyme assays, whilst five plugs were introduced to each conical flask for wood decay studies.

Enzyme production assays

The following growth conditions were used to test for lignocellulolytic enzyme production (Pointing, 2000):

Cellulose-azure agar – 1% (w/v) cellulose-azure (Sigma), 0.1% (w/v) mycological peptone (Oxoid), 0.01% (w/v) yeast extract (Difco), 1.5% (w/v) marine salts (Instant Ocean), 1.6% (w/v) agar (Difco). Cellulolysis was assessed by monitoring release of azure dye from cellulose-dye complex and diffusion into clear agar not containing cellulose-azure. Ligninolytic peroxidase production was indicated by subsequent decolorization of the azure dye.

Xylan agar – 1% (w/v) birchwood xylan (Sigma), 0.1% (w/v) mycological peptone, 0.01% (w/v) yeast extract, 1.5% (w/v) marine salts (Instant Ocean), 1.6% (w/v) agar. Zones of xylanolysis were visualized after flooding petri dishes with 0.25% (w/v) I_2 and KI solution. Xylan is the major component of hemicellulose and its hydrolysis in this study was used to indicate hemicellulolytic ability.

Poly R agar – 0.2% (w/v) glucose (Sigma), 0.02% (w/v) Poly R 478 (Sigma), 0.1% (w/v) mycological peptone, 0.01% (w/v) yeast extract, 1.5% (w/v) marine salts (Instant Ocean), 1.6% (w/v) agar. Production of lignin modifying enzymes was recorded as clearance of the Poly R dye.

Syringaldazine agar - 0.2% (w/v) glucose, 0.1% (w/v) mycological peptone, 0.01% (w/v) yeast extract, 1.5% (w/v) marine salts (Instant Ocean), 1.6% (w/v) agar. Formation of pink-purple zones around wells in the agar flooded with 0.1% (w/v) syringaldazine (Sigma) indicated laccase production.

Each treatment was repeated in triplicate with mean values used to determine enzyme production relative to control taxa. All incubations carried out at 25^{o}C in darkness.

Mass loss and lignin solubilization in wood

Untreated seasoned birch wood (*Betula* sp.) was chosen for its low extractive content and low durability. It was not possible to obtain mangrove wood of uniform quality for use in experiments and so the use of homogenous wood samples from a relatively benign wood species was preferred in order to limit bias due to substrate variability. Wood was cut into $1 \times 1 \times 2.5 cm^3$ blocks and leached in distilled water for 48 hours to remove water soluble components. Initial dry mass was determined after drying at 60°C for 2 days. Test blocks were then autoclaved and placed into 250ml Erlenmyer flasks that had been previously colonized for 2 week, on either the surface of 50ml agar (potato-dextrose agar, Difco) or submerged in 50ml liquid broth (potato-dextrose broth, Difco) supplemented with 1.5% w/v marine salts (Instant Ocean). Volume of liquid incubations was maintained by periodically adding sterile distilled water to flasks. These treatments were used to simulate exposed and submerged conditions respectively. High surface area to volume ratios of culture medium ensured aerated conditions were maintained throughout the experiment. Static incubation was chosen to minimize any bias due to physical abrasion of wood blocks that can occur during shaking incubation. Incubation was carried out at 25°C in darkness for 24 weeks. After incubation wood blocks were removed and final dry mass calculated after first removing fungal biomass from the surface of each wood block. Mass loss values were corrected for that recorded in uninoculated controls. Results were expressed as the mean of three replicates, plus/minus the standard deviation of the mean. Differences among treatments were analyzed by one-way ANOVA.

For chemical analysis wood blocks were ground in a hammer mill and the 250-500µm sized particle fraction prepared for analysis by ethanol-benzene and hot water extraction according to TAPPI T12 os-75 (Anon, 1975).

Determination of acid insoluble (Klason) lignin was carried out by measuring residual mass after digestion of wood particles in 72% H_2SO_4 according to TAPPI T222 os-98 (Anon, 1998), with uninoculated wood blocks serving as controls. The index of lignin solubilization (ILS) (percent lignin loss/percent mass loss) was calculated for each treatment. Results were expressed as the mean of three replicates, with the standard deviation of the mean shown by vertical error bars.

Results

Enzyme production assays (Table 1) revealed 89% of marine fungi were cellulolytic (crystalline cellulose utilization) and 84% were xylanolytic. Few isolates were cellulolytic only (13%) or xylanolytic only (9%). Some variation within genera was observed, with two strains of *Lulworthia* sp. and two species of *Marinosphaera mangrovei* variously able to produce xylanases and cellulases *in vitro* respectively. There were no obvious differences in enzyme production between ascomycetes or their anamorphs. The ability to produce enzymes involved in lignin degradation *in vitro* varied greatly between taxa used in this study. Of the anamorphs tested, 20% decolorized Poly R, whilst none decolorized Azure B and 30% oxidized syringaldazine. Many of the ascomycetes decolorized Poly R (77%), with 9% decolorizing Azure B and 17% oxidizing syringaldazine. The reactions of reference white-rot and soft-rot taxa were typical and confirmed the suitability of the assay medium for each test.

All marine fungi tested in this study caused mass loss in wood (Table 2), although values were generally extremely low (<5%) after 24 weeks exposure. Wood decay in exposed conditions was significantly higher ($P < 0.001$) than in submerged conditions for all groups, there was no significant difference between ascomycetes or anamorphic species ($P = 0.447$). The anamorphs, however, achieved significantly higher mass loss than ascomycetes in submerged conditions ($P < 0.01$). Six ascomycetes, *Ascocratera manglicola*, *Astrosphaeriella striatispora* (HKUCC 7651), *Cryptovalsa halosarceicola*, *Lignincola laevis* (HKUCC 6867), *Linocarpon bipolaris* and *Rhizophila marina*, caused mass loss in excess of 10% during exposed incubation. Mass loss values obtained for the three reference taxa were as expected under exposed conditions, with extensive mass loss by basidiomycetes and lower mass loss due to soft-rot of surface layers by *Chaetomium globosum*. During submerged exposure wood decay by basiodiomycetes was reduced 8-fold, but no significant change was observed for *Chaetomium globosum*.

Table 1. Production of wood decay enzymes *in vitro* by marine fungi.

	HKUCC Unless stated*	Cellulase	Xylanase	Poly R	Azure B	Syringaldazine
TERRESTRIAL FUNGI						
ASCOMYCETES						
Chaetomium globosum	4097	+	0	0	0	0
BASIDIOMYCETES						
Phanerochaete chrysosporium	IMI 284010	+	+	+	+	+
Pycnoporus sanguineus	IMI 307937	+	+	+	+	+
MARINE FUNGI						
ANAMORPHS						
Cytoplacosphaeria phragmaticola	6722	+	+	0	0	+
Cytospora rhizophorae	6012	0	0	0	0	0
Dactylaria sp.	6728	+	+	0	0	+
Dendryphiella salina	CY 2723	+	+	0	0	0
Periconia prolifica	6724	+	0	+	0	0
Phoma sp.	6725	+	0	0	0	0
Phomopsis sp.	6155	+	+	0	0	0
Trichocladium achrasporum	8266	0	0	0	0	+
Zalerion varium	5485	+	+	0	0	0
ASCOMYCETES						
Acrocordiopsis patilii	9145	+	0	0	0	+
Aigialus grandis	5796	0	+	+	0	0
Aniptodera salsuginosa	6729	+	+	+	0	0
Ascocratera manglicola	9174	+	+	0	0	0
Astrosphaeriella striatispora	5700	+	+	+	0	0
Astrosphaeriella striatispora	7651	+	+	+	0	+
Bathyascus grandisporus	6868	+	+	+	+	0
Botryosphaeria sp.	8019	+	+	0	0	0
Corollospora maritima	CY 1520	+	+	0	0	0
Cryptovalsa halosarceicola	9142	+	+	+	+	+
Dactylospora mangrovei	9141	+	+	+	0	NT
Eutypa sp.	CY GJ94	+	+	0	0	0
Ascosalsum cincinnatula	6731	+	+	+	0	0
Helicascus nypae	5788	+	+	0	0	0
Kallichroma tethys	6084	+	+	+	0	+
Hypocrea sp.	9144	+	0	0	0	0
Leptosphaeria sp.	6004	+	+	+	0	+
Lignicola laevis	6066	+	+	+	0	0

Table 1 continued. Production of wood decay enzymes in vitro by marine fungi.

	HKUCC Unless stated*	Cellulase	Xylanase	Poly R	Azure B	Syringaldazine
Lignincola laevis	6867	+	+	+	0	0
Lignincola laevis	6737	+	+	+	0	0
Linocarpon bipolaris	5790	+	+	+	0	0
Lulworthia grandispora	CY 1303	+	+	+	0	0
Lulworthia sp.	8054	+	+	+	0	0
Lulworthia sp.	8055	+	0	+	0	0
Marinosphaera mangrovei	8089	0	+	+	0	0
Marinosphaera mangrovei	6914	+	+	+	0	0
Massarina achostrichi	6727	+	+	0	0	0
Massarina thalassiae	9140	+	+	+	0	0
Massarina velatispora	5793	+	+	+	0	0
Neptunella longirostris	6712	+	+	+	0	0
Phragmitensis marina	6730	+	+	0	0	0
Quintaria sp.	6726	+	+	+	0	+
Rhizophila marina	9143	+	+	+	+	0
Salsuginea ramicola	6915	0	+	+	0	NT
Savoryella lignicola	9176	+	+	0	0	0
Verruculina enalia	6869	+	+	+	+	0

IMI = CABI Biosciences UK; CY = City University of Hong Kong Culture Collection.
+ denotes a positive reaction, 0 denotes a negative reaction, NT = not tested due to pigment production by the fungus.

The solubilization of lignin from wood blocks by those marine fungi causing more than 10% mass loss was assessed (Fig. 1). With the exception of *Lignincola laevis* (HKUCC 6867) all caused extensive lignin solubilization. The ILS values obtained show *Cryptovalsa halosarceicola* and *Linocarpon bipolaris* solubilized lignin from wood at a similar rate to other wood components. The high ILS (1.9) produced by *Rhizophila marina* suggests this fungus preferentially solubilized lignin over other wood components. Two other isolates, *Ascocratera manglicola* and *Astrosphaeriella striatispora* (HKUCC 7651), displayed an ILS value of 0.8 and 0.7 respectively. With the exception of *Ascocratera manglicola* all marine fungi causing more than 10% mass loss also produced LME's *in vitro*. *Lignincola laevis* (HKUCC 6867), however, was unable to solubilize lignin from wood in contrast to production of LME's in vitro. In the case of such ambiguities, data from direct

Table 2. Mass loss in wood under simulated exposed and submerged conditions by marine fungi after 24 weeks incubation.

Fungus	HKUCC Unless stated	Percent wood mass loss (± S.D. of the mean)	
		Exposed	**Submerged**
TERRESTRIAL FUNGI			
ASCOMYCETES			
Chaetomium globosum	4097	6.0±0.8	7.2±1.3
BASIDIOMYCETES			
Phanerochaete chrysosporium	IMI 284010	56.4±4	7.0±9.8
Pycnoporus sanguineus	IMI 307937	73.1±18.1	9.0±1.1
MARINE FUNGI			
ANAMORPHS			
Cytoplacosphaeria phragmaticola	6722	8.3 ±1.9	6.3 ±2.8
Cytospora rhizophorae	6012	2.3 ±0.4	1.5 ±0.2
Dactylaria sp.	6728	5.3 ±4	7.3 ±1.0
Dendryphiella salina	CY 2723	6.7 ±0.7	4.1 ±2.0
Periconia prolifica	6724	2.4 ±0.2	3.3 ±1.6
Phoma sp.	6725	3.5 ±0.4	6.6 ±1.5
Phomopsis sp.	6155	7.7 ±0.9	4.0 ±2.6
Trichocladium achrasporum	8266	5.2 ±1.3	1.6 ±0.5
Zalerion varium	5485	6.4 ±0.7	3.9 ±0.1
ASCOMYCETES			
Acrocordiopsis patilii	9145	ND	2.4 ±1.4
Aigialus grandis	5796	1.0 ±0.0	0.8 ±2.1
Aniptodera salsuginosa	6729	2.2 ±0.5	2.4 ±0.2
Ascocratera manglicola	9174	20.2 ±4.8	3.3 ±0.7
Astrosphaeriella striatispora	5700	9.6 ±1.0	4.7 ±3.9
Astrosphaeriella striatispora	7651	13 ±1.5	4.7 ±2.2
Bathyascus grandisporus	6868	3.3 ±1.4	1.6 ±0.7
Botryosphaeria sp.	8019	0.6 ±0.3	2.0 ±1.0
Corollospora maritima	CY 1520	5.3 ±0.3	2.6 ±1.5
Cryptovalsa halosarceicola	9142	20 ±13	4.5 ±3.4
Dactylospora mangrovei	9141	2.4 ±0.5	0.9 ±0.2
Eutypa sp.	CY GJ94	2.1 ±1.6	4.8 ±3.5
Ascosalsum cincinnatula	6731	3.1 ±0.9	1.0 ±0.4
Helicascus nypae	5788	1.1±0.6	0.6 ±0.1
Kallichroma tethys	6084	1.6 ±0.5	1.2 ±0.4
Hypocrea sp.	9144	3.2 ±0.5	4.0 ±1.8
Leptosphaeria sp.	6004	4.3 ±0.6	2.3 ±0.6
Lignicola laevis	6066	2.5 ±0.4	2.7 ±0.8
Lignincola laevis	6867	11.1 ±2.6	4.5 ±0.6
Lignincola laevis	6737	1.3 ±0.2	1.0 ±0.4
Linocarpon bipolaris	5790	11.8 ±2.1	6.7 ±1.2

Table 2 continued. Mass loss in wood under simulated exposed and submerged conditions by marine fungi after 24 weeks incubation.

Fungus	HKUCC Unless stated	Percent wood mass loss (± S.D. of the mean)	
		Exposed	Submerged
Lulworthia grandispora	CY 1303	5.5 ±0.5	2.9 ±1.6
Lulworthia sp.	8054	8.1 ±1.4	3.0 ±0.5
Lulworthia sp.	8055	3.5 ±0.0	2.5 ±0.1
Marinosphaera mangrovei	8089	6.4 ±1.1	2.6 ±0.5
Marinosphaera mangrovei	6914	3.8 ±0.6	4.9 ±3.1
Massarina achostrichi	6727	4.1 ±0.2	1.1 ±0.2
Massarina thalassiae	9140	0.4 ±0.4	0.1 ±0.9
Massarina velatispora	5793	4.8 ±0.5	1.8 ±2.0
Neptunella longirostris	6712	8.3 ±1.1	2.3 ±0.4
Phragmitensis marina	6730	1.2 ±0.3	4.0 ±2.1
Quintaria sp.	6726	6.8 ±0.6	1.6 ±0.9
Rhizophila marina	9143	12.9 ±4.8	2.6 ±0.5
Salsuginea ramicola	6915	ND	ND
Savoryella lignicola	9176	8.1 ±0.6	5.4 ±1.8
Verruculina enalia	6869	6.4 ±0.5	4.0 ±1.6

IMI = CABI Biosiences UK; CY = City University of Hong Kong Culture Collection.
ND = not determined due to poor growth of fungus.

measurement of lignin solubilization or mineralization is regarded as more indicative of fungal ability.

Discussion

The high incidence of cellulase and xylanase production indicates that a large number of marine ascomycetes and anamorphic isolates are at least physiologically capable of wood decay. This is unsurprising since cellulose and hemicellulose serve as nutritional carbon sources in wood (Pointing and Hyde, 2000). There were no obvious differences in enzyme production between ascomycetes and their anamorphs. Other studies have also qualitatively demonstrated cellulolytic activity among a wide range of marine fungi (Gessner, 1980; Leightley, 1980; McDonald and Speedie, 1982; Rohrmann and Molitoris, 1992; Raghukumar *et al*., 1994; Pointing *et al*., 1998, 1999). The ability of marine fungi to produce enzymes involved in lignin degradation *in vitro* is less well studied, and results from this study varied greatly between taxonomic groups. Anamorphic fungi were incapable of peroxidase or laccase

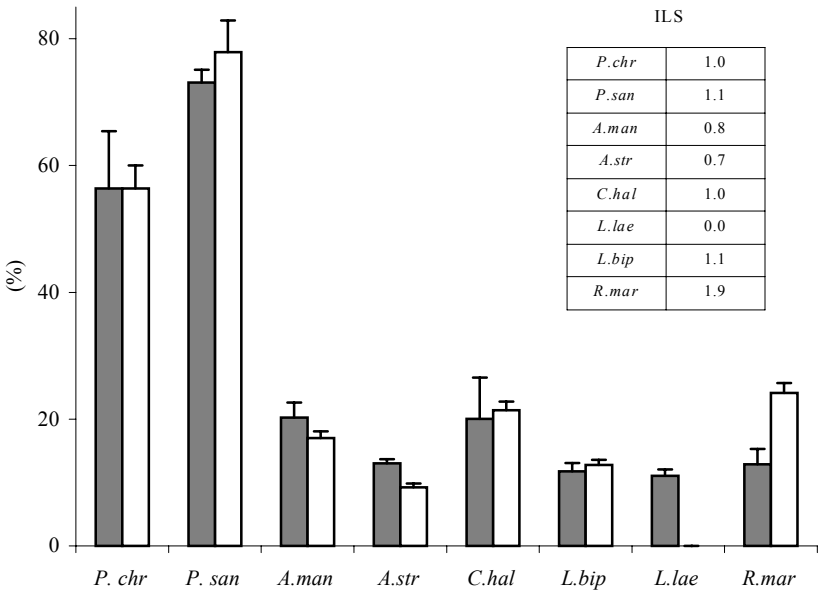

Fig. 1. Mass loss and lignin solubilization in wood by selected marine ascomycetes after 24 weeks exposed incubation. Terrestrial controls: *P.chr*, *Phanerochaete chrysosporium*; *P.san*, *Pycnoporus sanguineus*. Marine ascomycetes: *A.man*, *Ascrocratera manglicola*; *A.str*, *Astrosphaeriella striatispora* (HKUCC 7651); *C.hal*, *Cryptovalsa halosarceicola*; *L.lae*, *Lignincola laevis* HKUCC 6867); *L.bip*, *Linocarpon bipolaris*; *R.mar*, *Rhizophila marina*. ILS = index of lignin solubilization. Shaded = mass loss; unshaded = lignin solubilization. Error bars represent standard deviation of the mean.

type ligninolytic enzyme production, suggesting they are non-ligninolytic. The reason for this difference between sexual and asexual states is unclear. In the case of marine ascomycetes, laccase and/or Mn-dependant peroxidase are probably the most commonly secreted enzymes involved in lignin breakdown, since none of the isolates were capable of Azure B decolorization. Both these enzymes are known to mediate lignin mineralization (Thurston, 1994; Orth and Tien, 1995) and so indicates a possible physiological capability for lignin degradation by these fungi. The inability of apparently ligninolytic marine fungi to produce lignin peroxidase was also recorded by Raghukumar *et al*. (1994). A demonstrated ability to mineralize ^{14}C-labelled lignin is necessary to confirm ligninolytic ability by marine fungi.

That few fungi in this study were capable of effecting significant mass loss in Birch wood during a 24-week incubation strongly indicates that many fungi are unlikely to be important in terms of wood decay in the marine environment. The use of birch wood rather than mangrove wood is highly unlikely to result in underestimates of wood decay ability, since birch is known to have an extremely low extractive content (responsible for decay resistance in wood) and low durability. Those taxa capable of more aggressive wood decay in this study are likely to play a key role, as mass loss data for marine lignocellulosic materials are generally much higher, with values for mangrove wood and marine grasses of up to 20 % and 51 % respectively under similar conditions (Vrijmoed *et al*., 1999). The possibility of substrate specificity by marine fungi was suggested by Hyde (1986), based on observed colonization patterns of mangrove wood in the tropics. Furthermore, Mouzouras (1989b) demonstrated that the basidiomycete *Halocyphina villosa* caused mass loss only in certain mangrove wood species and not others, indicating specificity also among naturally available wood substrates. The significantly higher mass loss values obtained for wood incubated in simulated exposed conditions strongly suggests that fungal decay is more significant in degradation of substrates in the upper intertidal region. Decay probably occurs at much slower rates when wood is submerged, although this must be offset against the reduced role of fungi in wood decay where marine borers may be active (Eltringham, 1971).

Five marine ascomycetes were demonstrated to solubilize significant amounts of lignin from wood in this study. The ILS values obtained show *Cryptovalsa halosarceicola* and *Linocarpon bipolaris* solubilized lignin from wood at a similar rate to other wood components, a decay strategy only previously associated with simultaneous white-rot basidiomycetes. The high ILS produced by *Rhizophila marina* suggest this fungus is capable of preferential white-rot, where lignin is solubilized more rapidly than other wood components. Two other isolates, *Ascocratera manglicola* and *Astrosphaeriella striatispora* (HKUCC 7651), displayed ILS values similar to terrestrial ligninolytic ascomycetes (Worrall *et al*., 1997). It is interesting that the strongly lignin-solubilizing isolates *Astrosphaeriella striatispora* and *Linocarpon bipolaris* have only been previously recorded growing on *Nypa* palm, a poorly lignified substrate, within intertidal mangroves (Yanna *et al*., 2002). Only one other study reports lignin solubilization from wood by a marine fungus, at relatively low levels (<12%) by a species of *Zalerion maritimum* (anamorphic) after 6 months exposure (Henningsson, 1976).

The *in vitro* mineralization of radiolabelled synthetic lignin to CO_2 has been demonstrated for 12 marine strains including several ascomycetes and

anamorphic fungi, plus the basidiomycete *Nia vibrissa*, albeit at low levels (<5% in 60 days) (Sutherland *et al*., 1982). More aggressive degradation of synthetic lignin was recorded for a terrestrial basidiomycete *Flavodon flavus* (24% in 24 days) isolated from decaying seagrass in the intertidal region (Raghukumar *et al*., 1999). The enzymes lignin peroxidase, Mn dependant peroxidase and laccase were responsible for lignin mineralization by this isolate. Mineralization was optimal under non-saline conditions, but also occurred significantly under conditions simulating full strength seawater (approx. 20% in 24 day). It is not known if this fungus is capable of wood decay and lignin solubilization in a wood substrate. The data from our study shows a dramatic reduction in wood decay ability of terrestrial basidiomycetes when submerged, and so it is likely that lignin mineralization rates would be considerably reduced during high tides. Despite this, evidence suggests that certain terrestrial basidiomycetes can tolerate saline growth conditions *in vitro* (Castillo and Demoulin, 1997), and we have shown in this study that they are capable of wood decay in submerged saline conditions *in vitro* at rates similar to marine fungi. The relative lack of inhibition in wood decay during submerged incubation for *Chaetomium globosum* is consistent with the substrate recurrence of soft-rot fungi for wet or waterlogged wood (Zabel and Morrel, 1992).

This study conclusively demonstrates the ability of certain marine ascomycetes to solubilize lignin from wood with ILS values equivalent or better than known terrestrial white-rot basidiomycetes, suggesting that marine ascomycetes carry out a 'white-rot like' role in marine environments. Terrestrial white-rot fungi have also been demonstrated to mineralize a range of organic pollutants via their ligninolytic enzyme system (Reddy, 1995; Pointing, 2001). It is therefore conceivable that marine fungi are also involved in ameliorating pollution within estuarine environments such as mangroves, which often receive a high pollution input. This places a high value on the importance of fungi in coastal ecology.

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References

Anon. (1975). Preparation of wood for chemical analysis. In: *Technical Association of the Pulp and Paper Industry standard T12 os-75*.

Anon. (1998). Acid-insoluble lignin in wood and pulp. In: *Technical Association of the Pulp and Paper Industry standard T222 os-98*.

Castillo, G. and Demoulin, V. (1997). NaCl salinity and temperature effects on growth of three wood-rotting basidiomycetes from Papua New Guinea coastal forest. Mycological Research 101: 341-344.

Eaton, R.A. and Hale, M.D.C. (1993). *Wood: decay*, *pests and protection*. Chapman & Hall, London.

Eltringham, S.K. (1971). Marine borers and fungi. In: *Marine Borers*, *Fungi and Fouling Organisms of Wood* (eds. E.B.G. Jones and S.K. Eltringham) OECD, Paris: 327-335.

Gessner, R.V. (1980). Degradative enzyme production by salt-marsh fungi. Botanica Marina 23: 133-139.

Henningsson, M. (1976). Degradation of wood by some fungi from the Baltic and West coast of Sweden. Material Organismen Beih 3: 409-519.

Hyde, K.D. (1986). Frequency of occurrence of lignicolous marine fungi in the tropics. In: *The Biology of Marine Fungi*. (ed. S.T. Moss) Cambridge University Press, Cambridge: 311-322.

Hyde, K.D. (1991). Fungal colonization of *Rhizophora apiculata* and *Xylocarpus granatum* poles in Kampong Kapok mangrove, Brunei. Sydowia 43: 31-38.

Jones, E.B.G. and Alias, S.A. (1997). Biodiversity of mangrove fungi. In: *Biodiversity of Tropical Microfungi*. (ed. K.D. Hyde) Hong Kong University Press, Hong Kong: 71-92.

Leightley, L.E. (1980). Wood decay activities of marine fungi. Botanica Marina 23: 387- 395.

Leightley, L.E. and Eaton, R.A. (1979a). Micromorphology of wood decay by marine microorganisms. In: *Biodeterioration 4* (eds. T.A. Oxley, D. Allsopp and G. Becker). Pitman, London: 83-88.

Leightley, L.E. and Eaton, R.A. (1979b). *Nia vibrissa* – a marine white rot fungus. Transactions of the British Mycological Society 73: 35-40.

MacDonald, M.J. and Speedie, M.K. (1982). Cell-associated and extracellular celluloytic enzyme activity in the marine fungus *Dendryphiella arenaria*. Canadian Journal of Botany 60: 838-844.

Mouzouras, R. (1986). Patterns of timber decay caused by marine fungi. In: *The Biology of Marine Fungi*. (ed. S.T. Moss) Cambridge University Press, Cambridge: 341- 353.

Mouzouras, R. (1989a). Soft rot decay of wood by marine fungi. Journal of the Institute of Wood Science 11: 193-201.

Mouzouras, R. (1989b). Decay of mangrove wood by marine fungi. Botanica Marina 32: 65-69.

Orth, A.B. and Tien, M. (1995). Biotechnology of lignin degradation. In: *The Mycota II Genetics and Biotechnology* (eds. K. Esser and P.A. Lemke) Springer-Verlag, London: 287-302.

Pointing, S.B., Vrijmoed, L.L.P. and Jones, E.B.G. (1998). A qualitative assessment of lignocellulose degrading enzyme activity in marine fungi. Botanica Marina 41: 293-298.

Pointing, S.B., Buswell, J.A., Jones, E.B.G. and Vrijmoed, L.L.P. (1999). Extracellular cellulolytic enzyme profiles of five lignicolous mangrove fungi. Mycological Research 103: 696-700.

Pointing, S.B. (2000). Lignocellulolytic enzyme assays. In: *Marine Mycology - A Practical Approach* (eds. K.D. Hyde and S.B. Pointing). Fungal Diversity Research Series 1: 137-157.

Pointing, S.B. and Hyde, K.D. (2000). Lignocellulose-degrading marine fungi. Biofouling 15: 221- 229.

Pointing, S.B. (2001). Feasibility of bioremediation by white-rot fungi. Applied Microbiology and Biotechnology 57: 20-33.

Raghukumar, C., Raghukumar, S., Chinnaraj, A., Chandramohan, T.M., D'Souza and Reddy, C.A. (1994). Laccase and other lignocellulose modifying enzymes of marine fungi isolated from the coast of India. Botanica Marina 37: 515-523.

Raghukumar, C., D'Souza, T.M., Thorn, G.R. and Reddy, C.A. (1999). Lignin modifying enzymes of *Flavodon flavus*, a basidiomycete isolated from a coastal marine environment. Applied and Environmental Microbiology 65: 2103-2111.

Reddy, C.A. (1995). The potential for white-rot fungi in the treatment of pollutants. Current Opinion in Biotechnology 6: 320-328.

Risna, R.A. and Suhirman (2002). Ligninolytic enzyme production by *Polyporaceae* from Lombok, Indonesia. Fungal Diversity 9: 123-134.

Rohrmann, S. and Molitoris, H.P. (1992). Screening of wood-degrading enzymes in marine fungi. Canadian Journal of Botany 70: 2116-2123.

Sarma, V.V. and Hyde, K.D. (2001). A review on frequently occurring fungi in mangroves. Fungal Diversity 8: 1-34.

Sarma, V.V. and Vittal, B.P.R. (2001). Biodiversity of manglicolous fungi on selected plants in the Godavari and Krishna deltas, east coast of India. Fungal Diversity 6: 115-130.

Singh, A.P. and Butcher, J.A. (1991). Bacterial degradation of wood cell walls: a review of degradation patterns. Journal of the Institute of Wood Science 12: 143-157.

Sutherland, J.B., Crawford, D.L. and Speediem, M.K. (1982). Decomposition of ^{14}C-labeled maple and spruce lignin by marine fungi. Mycologia 74: 511-513.

Thurston, C.F. (1994). The structure and function of fungal laccases. Microbiology 140: 19-26.

Vrijmoed, L.L.P., Pointing, S.B. and Sadaba, R.B. (1999). Decomposition of different substrata in marine habitats. In: *Abstracts of the 7th International Marine and Freshwater Mycology Symposium*, *Hong Kong*. City University of Hong Kong, Hong Kong: 38.

Worrall, J.J., Anagnost, S.E. and Zabel, R.A. (1997). Comparison of wood decay among diverse lignicolous fungi. Mycologia 89: 199-219.

Uraiuj, C., Khanongnoch, C. and Lumyong, S. (2003). Ligninolytic enzymes from tropical endophytic *Xylariaceae*. Fungal Diversity 13: 209-219.

Yanna, Ho, W.H. and Hyde, K.D. (2002). Fungal succession on fronds of *Phoenix hanceana* in Hong Kong. Fungal Diversity 10: 185-211.

Zabel, R.A. and Morrell, J.J. (1992). *Wood Microbiology: decay and its prevention*. Academic Press, California.

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